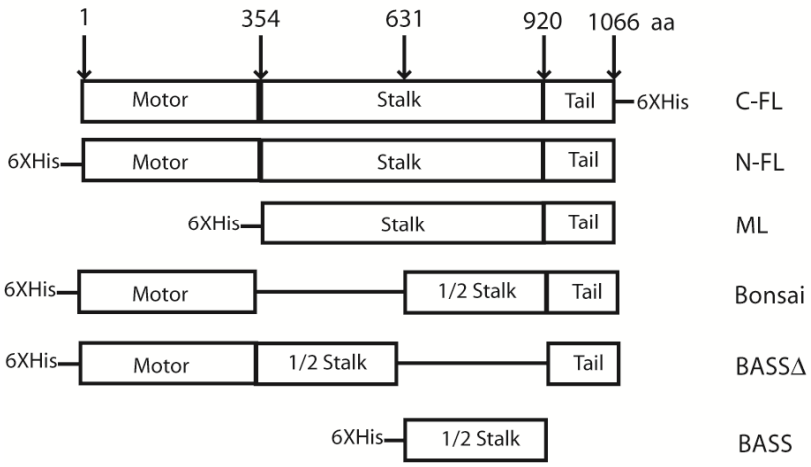
Supplementary Figures

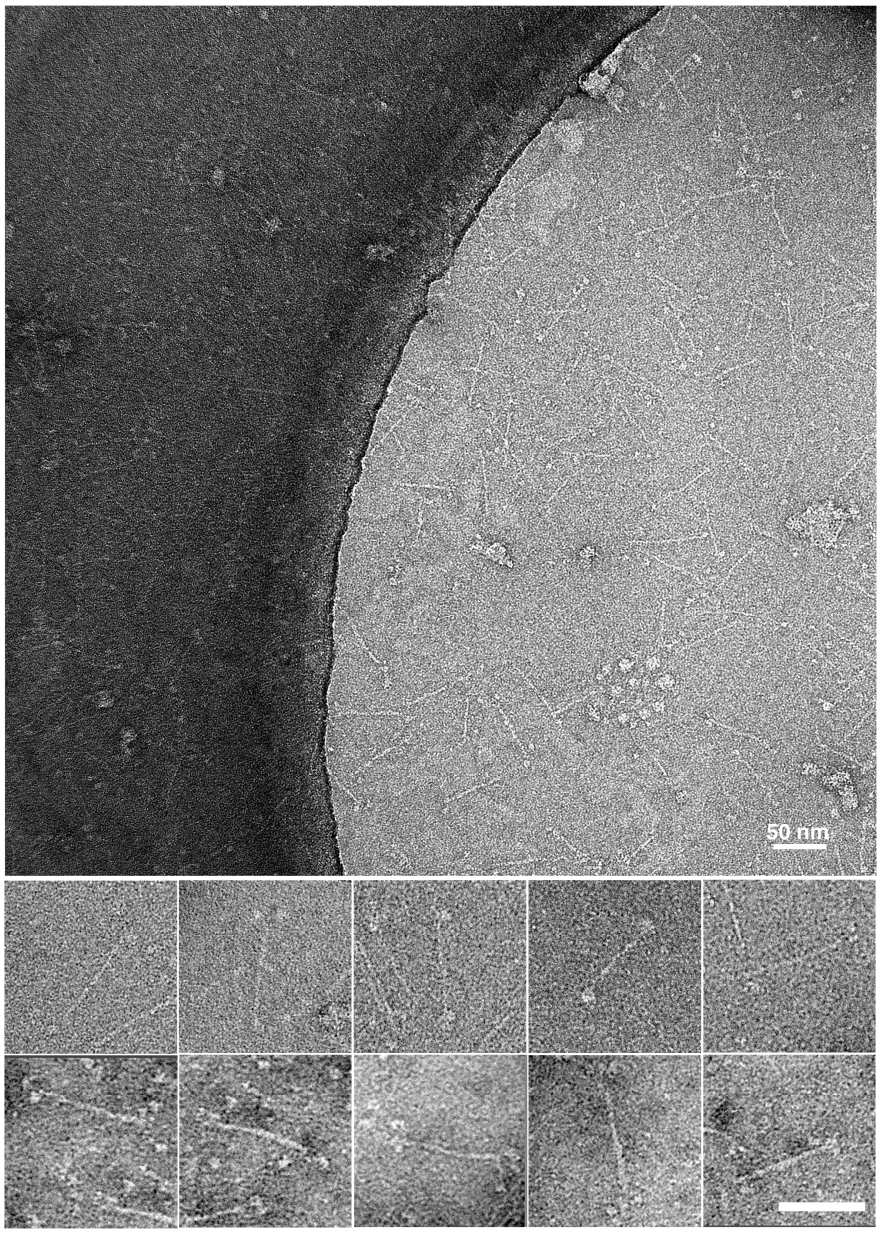
a



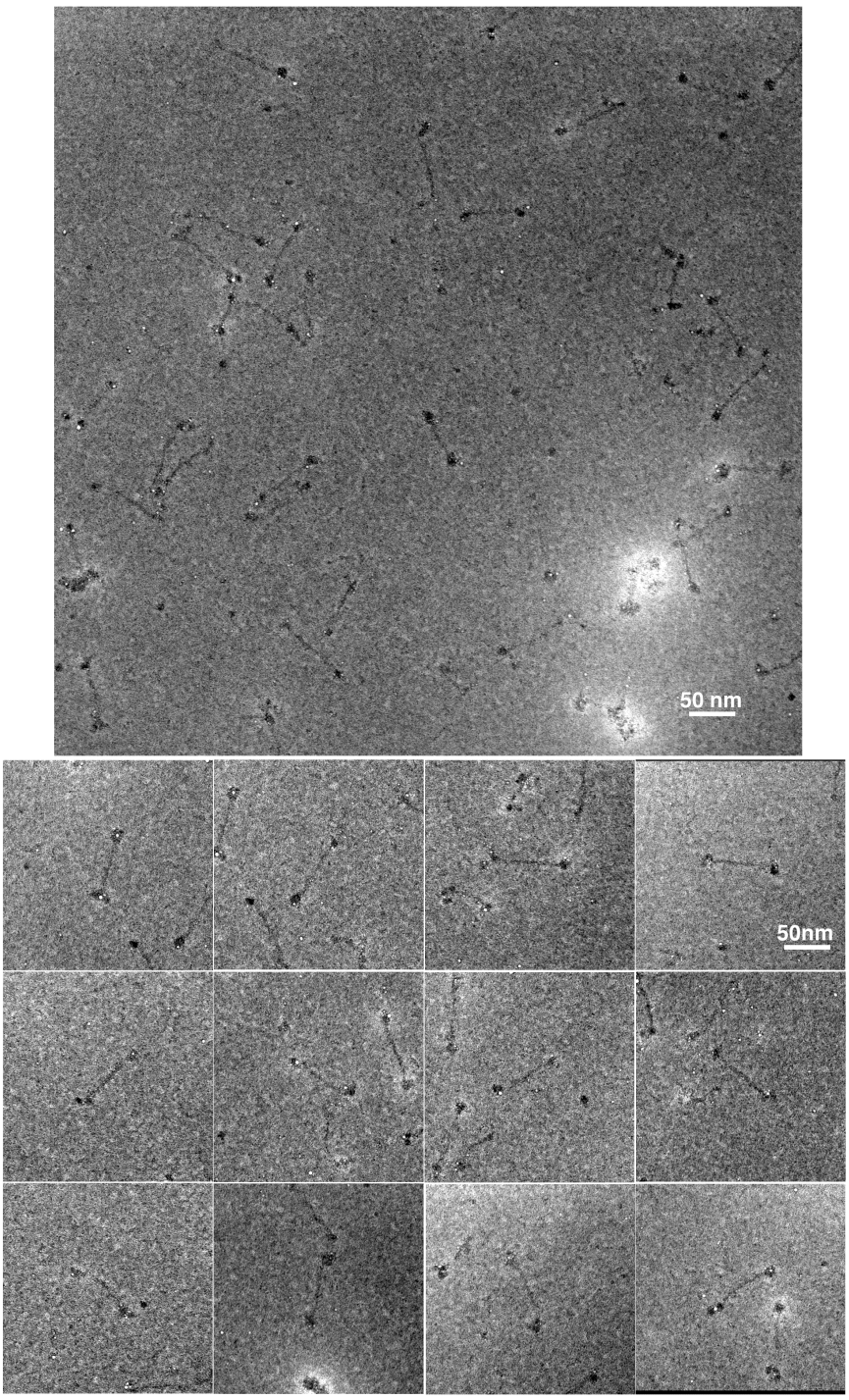
b

Measured	*Length (nm)*	*n*
Full length	79.4±7.9	1371
Motorless	73.4±12.0	1379
Full length rod	57.1±7.1	1215
Rod diameter	3.1±0.6	924
Motor (major axis)	8.1±1.7	472
Motor (minor axis)	5.5±1.1	472
BASS	31.8±5.6	1103
Bonsai FL	38.1±4.6	500
Full decoration statistics for full length Kinesin-5	*n*	
Decorated at both ends	530	
Decorated at one end	110	
Undecorated	23	

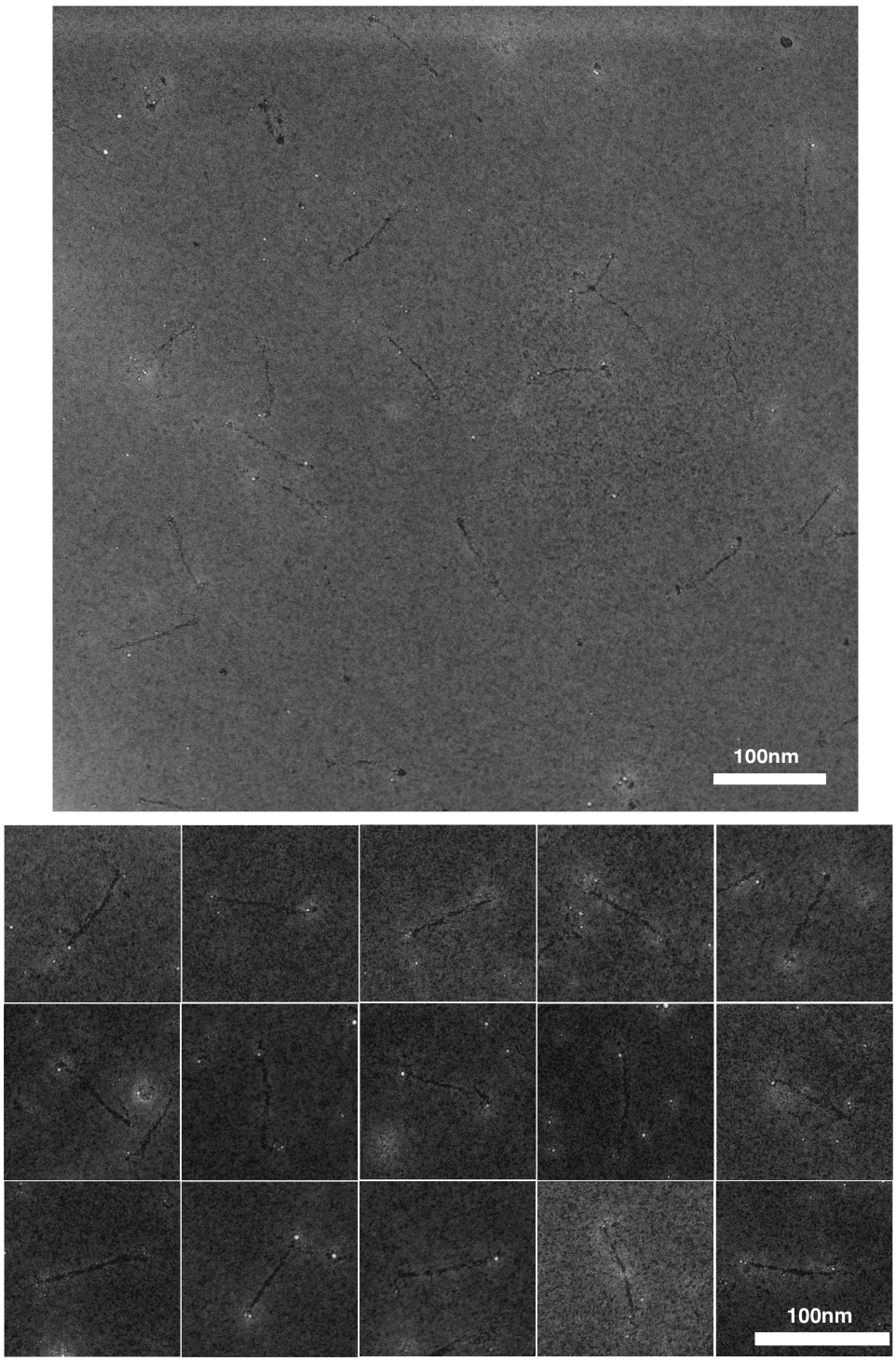
Supplementary Figure S1. Map of constructs analyzed and the statistics of EM analysis. a. Map showing 6xHis-tagged Kinesin-5 (N-terminal HisTagged (N-FL), C-terminal HisTagged (C-FL), motorless Kinesin-5 (ML), bipolar assembly domain (BASS), and Bonsai. b. Table showing the dimensions of the constructs and the described domains found by EM measurements and statistical analysis. Second part of table shows STEM statistics of 2 nm Ni-NTA nanogold decorating N-terminal full length Kinesin-5. Bidecorated Kinesin-5 has at least one gold particle associated with the head group on both sides of the central rod. Monodecorated has at least one gold particle associated with the head group on one side of the central rod. Undecorated has no gold particles associated with the head groups.



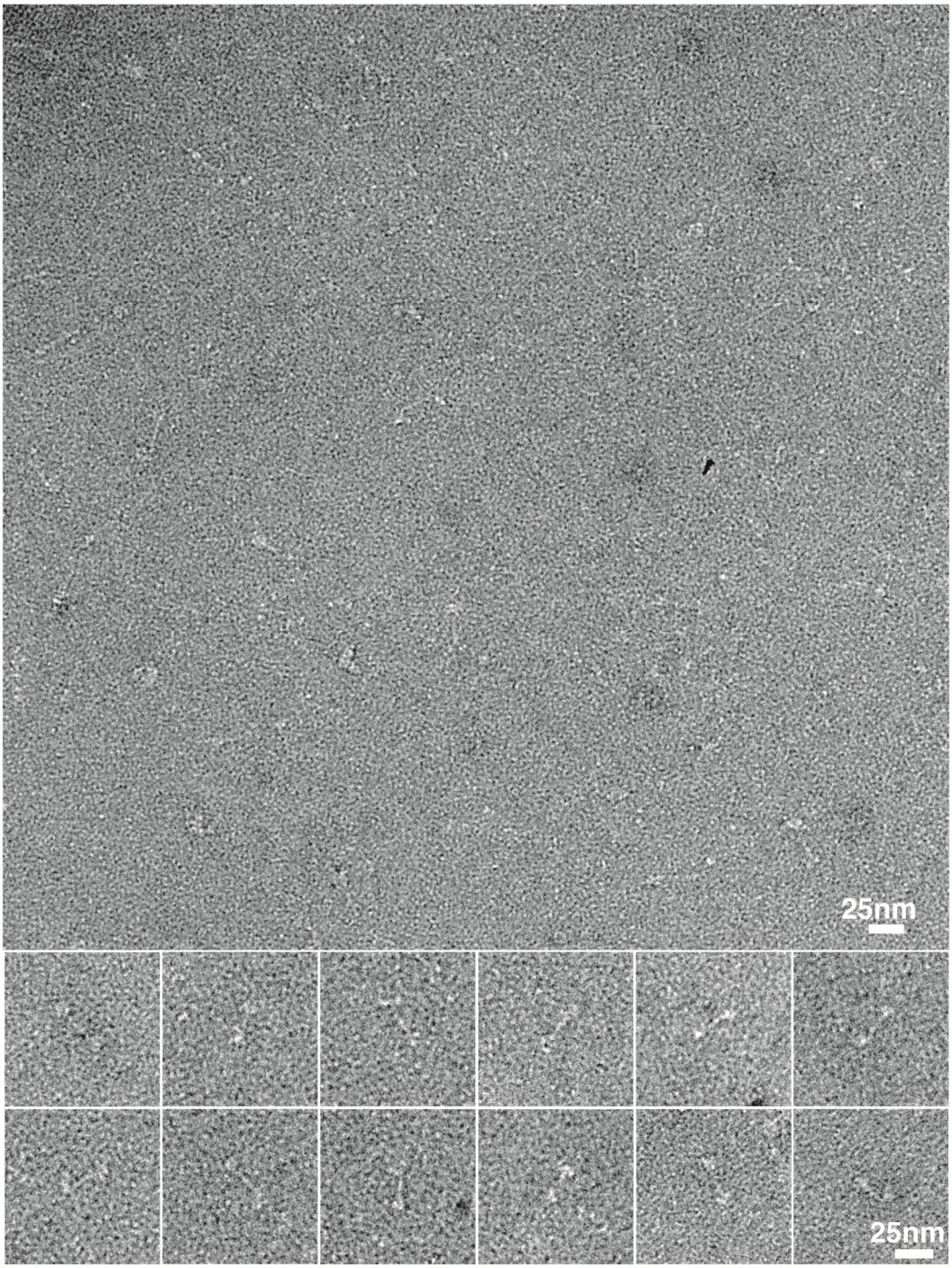
Supplementary Figure S2. TEM of N-terminal 6xHis tagged Full length KLP61F (N-FL). a. Large image is a representative view of uranyl formate negative stained protein on thin carbon (light with good contrast) spanning a holy carbon support (dark-poor contrast). b. Small images in two rows are selected N-FL particles. Scale bars = 50 nm.



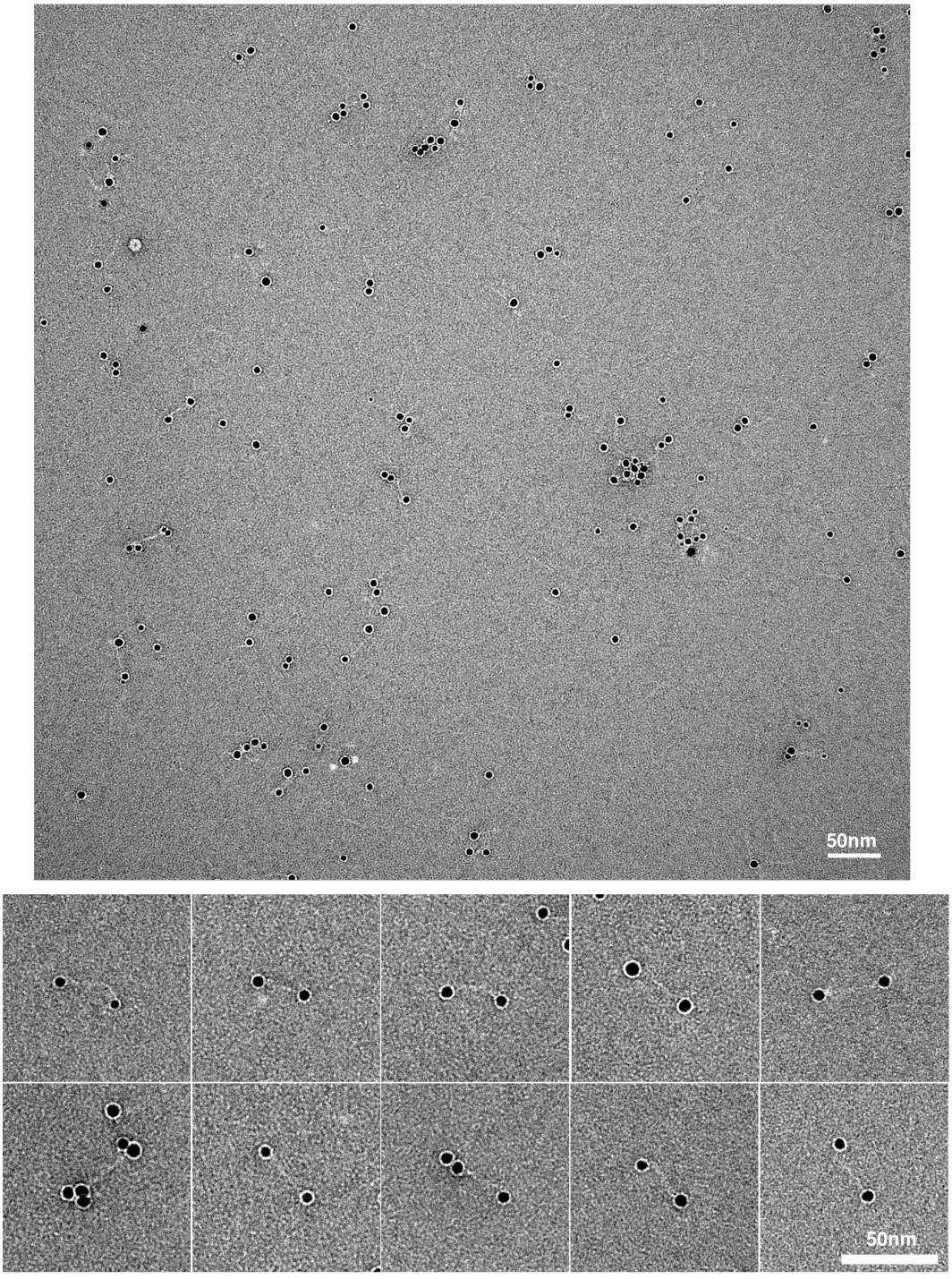
Supplementary Figure S3. STEM of C-FL decorated with 2 nm Ni-NTA nanogold. a. Large image is a representative view of uranyl formate negative stained C-FL decorated with 2 nm Ni-NTA nanogold. b. Small images in three rows are selected C-FL particles decorated with 2 nm nanogold. Scale bars = 50 nm.



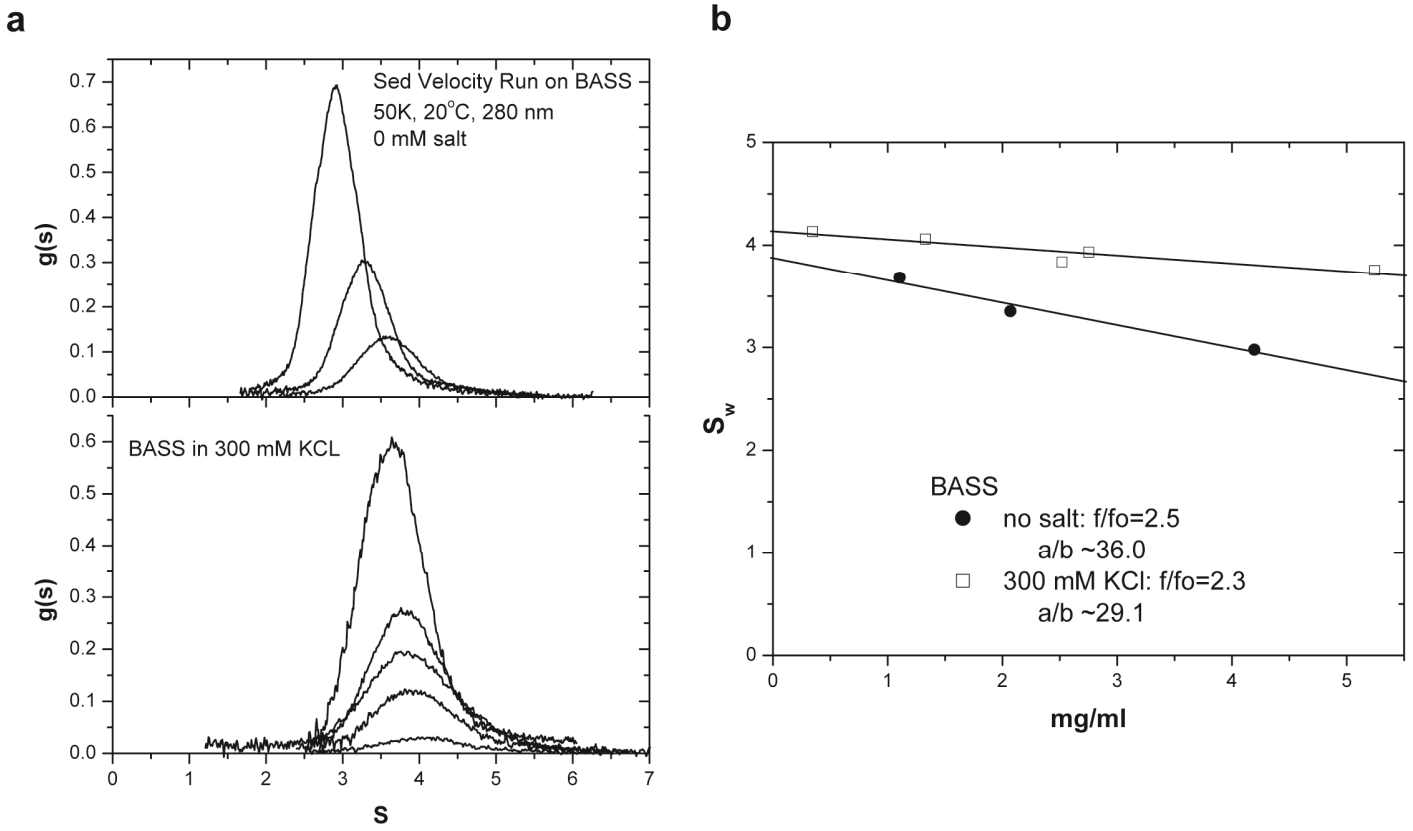
Supplementary Figure S4. STEM of ML decorated with 2 nm Ni-NTA nanogold. a. Large image is a representative view of uranyl formate negative stained ML decorated with 2 nm Ni-NTA nanogold. b. Small images in three rows are selected ML particles decorated with 2 nm nanogold. Scale bars = 100 nm.



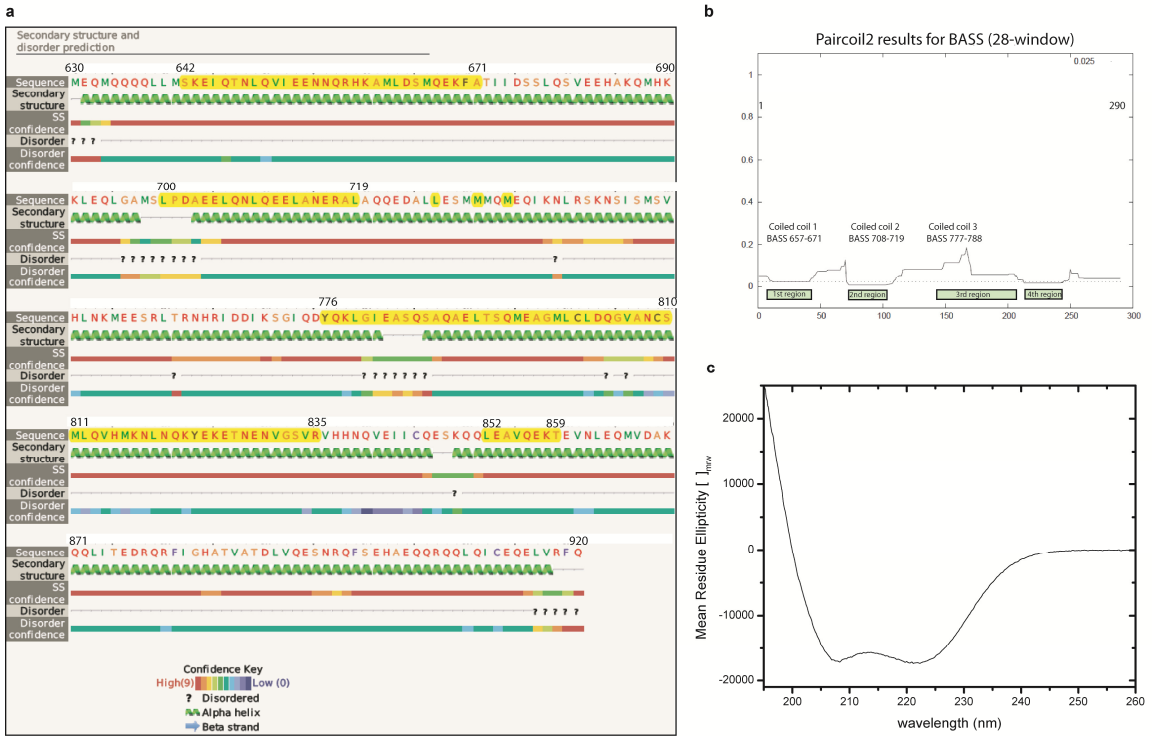
Supplementary Figure S5. TEM of undecorated Bonsai construct a. Large image is a representative view of uranyl formate negative stained Bonsai b. Small images in two rows are selected Bonsai particles. Scale bars = 25 nm.



Supplementary Figure S6. TEM of BASS decorated with 5 nm Ni-NTA nanogold. a. Large image is a representative view of uranyl formate negative stained BASS decorated with 5 nm Ni-NTA nanogold. 5 nm gold is easily detected in negative stain, however, it obscures the ends of the protein. b. Small images in two rows are selected BASS particles decorated with 5 nm nanogold. Scale bars = 50 nm & 25 nm, respectively.



Supplementary Figure S7. Analytical Ultracentrifugation of BASS for determination of hydrodynamic parameters. a. Plot of the g(s) curves generated from three (upper) and five (lower) samples at different concentrations of BASS. The upper plot shows sedimentation velocity runs of BASS in a buffer containing no salt and the lower plot shows one with the inclusion of 300mM KCl. Sedimentation velocity data were collected at 50K rpm, 20ºC at a wavelength of 280 nm. b. The data in panel a was analyzed to generate Sw (the weight average sedimentation coefficient) values. The inset data gives the f/f_o and a/b (axial) ratio for BASS.



Supplementary Figure S8. Secondary and coiled coil prediction and CD spectra of BASS domain. a. Schematic representation of the secondary structure prediction from Phyre2 server (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) from Imperial College London. The probabilities of the structure predicted and the disorder prediction confidence are given in a color coded way underneath the sequence. The BASS domain cysteine mutants analyzed (in four different regions) by EPR are highlighted with yellow. b. Lupas Plot for prediction of coiled coil regions in BASS domain. The probability of having a coiled coil changes along the sequence as given as *P*-score. The regions where the score is lower than set score (=0.025), below the dashed line are more likely to contain coiled coils. Four regions analyzed by EPR spectroscopy are also marked with boxes and corresponding names. Results of the EPR show that there are three clear coiled coils as marked on the Lupas Plot. The residues within the coiled coil regions are also stated. c. CD spectrum of BASS domain. CD spectra were collected on a Jasco J720 in 300 mM NaCl buffer, 0.2 mm path cell; mean residue MW = 115.8 was used to correct data to mean residue ellipticity. Note the classic minima for α-helix at 222 and 208 nm.

Supplementary Methods.

Cloning

Cloning of the genes was done using the Gateway system (Invitrogen) following the instructions of the manufacturer. In summary the genes were cloned using PCR cloning using attB1 and attB2 primer set designed for the corresponding gene and or domain. The genes (without introns) were inserted into entry plasmid pENTRY221 using BP reaction. The entry clones which were sequenced and confirmed to have the correct sequence were transferred into baculovirus expression vector pDEST8. The PDEST clones were transformed into *E.coli* DH10Bac containing the bacmid and helper plasmid. In vivo recombination of the pDEST vector containing the gene forms the bacmid for expression. Bacmids were analyzed for the presence of the gene of interest by doing PCR using M13 forward (5'GTTTTCCCAGTCACGAC3') and M13 reverse primers (5'CAGGAAACAGCTATGAC3'), further verification of the identity of the insert was done by sequencing the PCR product with primers within the desired insert sequence.

For making BASSΔ and Bonsai constructs QuikChange Site Directed Mutagenesis kit (Stratagene) was used. The primers were designed according to the manual of the manufacturer. The pDEST vector carrying full length Kinesin-5 was used as the template for the reactions. For deletions of size larger than 500bp the primers were designed so that the deletion could be done in two rounds of QuikChange reactions. Both the resulting constructs were then sequenced and verified for the deletion of the desired region. The destination vectors containing BASSΔ and Bonsai were prepared for baculovirus expression as described in the previous paragraph.

BASS constructs for EPR study, each containing a single Cys residue were obtained using QuikChange Site Directed Mutagenesis Kit. There were 4 Cys in the original BASS sequence. These were first mutated into Ser to make a BASS no cysteine (BASSNoC) mutant in pDEST8 vector. This mutant construct was used as the template for introducing all the other Cys mutations. Primers were designed according to the manufacturer's manual. All the constructs obtained at the end of the mutation reactions and basic molecular biological steps were verified by sequencing the whole insert. The destination vectors were then prepared for baculovirus expression.